

# Competitive Technology/ Detailed Technical Assessment: Process Evaluation/Development

Jim McMillan

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## Major Steps in an Enzymatic Process

Lignocellulose  
Feedstock  
Collection and  
Delivery

Pre-processing

Pretreatment  
(hemicellulose  
extraction)

Conditioning

Enzymatic  
cellulose  
saccharification

Biomass  
sugar  
fermentation

Beer Slurry  
to Ethanol  
and Solids  
Recovery

Many options exist for  
each of these steps....  
....and there are many  
interactions to consider

## Process Development Options



## Critical Success Factor for Pioneer Plants

✍ Accurately estimate cost and performance!\*

- Plant cost growth strongly correlated with:
  - Process understanding (integration issues)
  - Project definition (estimate inclusiveness)
- Plant performance strongly correlated with:
  - Number of new steps
  - % of heat and mass balance equations based on data
  - Waste handling difficulties
  - Plant processes primarily solid feedstock

\* “Understanding Cost Growth and Performance Shortfalls in Pioneer Process Plants”, a study by the Rand Corp. for DOE (1981)

# Technology Selection

✍ Focus on pretreatment and fermentation strains.....  
Final enzyme characteristics remain unknown

✍ Apply 2-step screening methodology

- 1<sup>o</sup> screen: Reported efficacy
  - Obtained from the literature or personal communications
- 2<sup>o</sup> screen: Quantitative performance and readiness
  - Mass balanced performance data?
  - Process models show favorable economic potential?
  - Showstopper regulatory/permitting issues?
  - Ready for pilot testing?
  - Available for third party commercialization?

## Part II: Presentations

✍ Feedstock – John Sheehan

✍ Pretreatment – Dan Schell

✍ Enzymes – Jim McMillan

✍ Genencor – Bill Dean and Mike Knauf

✍ Novozymes – Joel Cherry

✍ Fermentation strains – Kiran Kadam

## Today

- Project Overview
- Market Assessment
- Technical and Economic Analysis
- Life Cycle Analysis

### **Feedstock**

- Pretreatment
- Enzyme
- Fermentation Microorganism
- Business plan
- High-level Stage 3 plan

## Feedstock

Agricultural residues like corn stover are the cornerstone of DOE's first generation of sugar platform biorefineries.

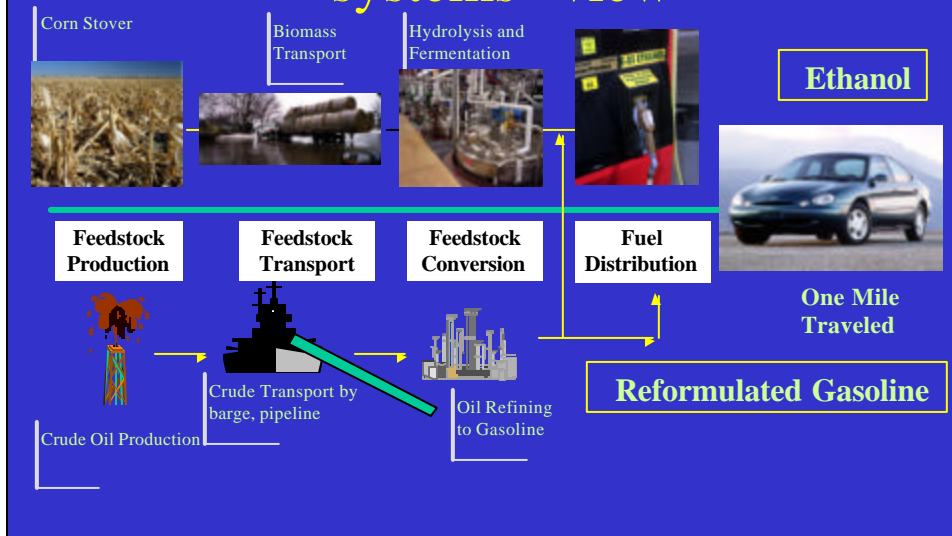


## Critical success factors

- Adequate supply of stover
- Ability to sustainably collect stover
  - Soil health
  - Environmental issues
  - Economic impacts of stover collection and use
- Ability to collect stover cost-effectively
  - Stover costs contribute significantly to ethanol cost



## We have used LCA to keep a “systems” view



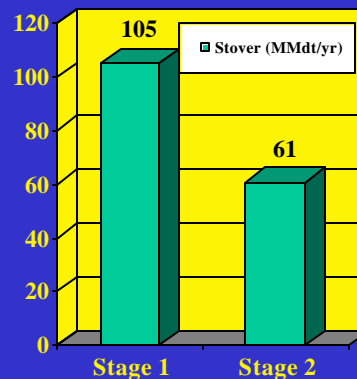
## Stage 2 feedstock work involved experts from many organizations within DOE and USDA

- ORNL
  - Collection logistics, resource assessment and macroeconomic impacts
- USDA ARS
  - Soil science research and modeling
- USDA NRCS
  - Soil conservation issues
- USDA Office of Energy
  - Agronomics of bioenergy supply

## Stage 2 findings

### Available supply of stover

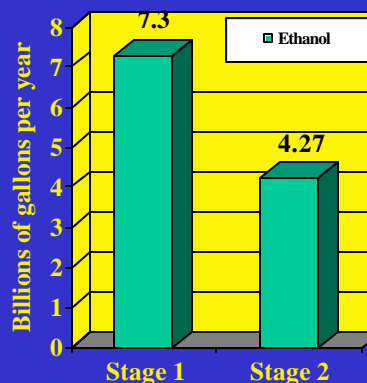
- Stage 1 analysis estimated 105 MMdt per year of stover in top 10 corn states
- Stage 2 analysis has further refined that estimate at 61 MMdt
- These are conservative estimates based on no changes in current farm practices



## Stage 2 findings

### Available supply of stover

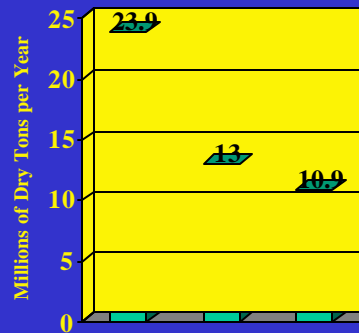
- Potential ethanol supply and demand are well matched
  - The available market for stover derived ethanol is at least 3 billion gallons beyond the future supply from corn grain
  - Stage 2 estimates of supply confirm the ability to support this market



## Stage 2 findings

### More rigor in assessment of sustainable stover supply

- The stage 1 preliminary analysis for Iowa showed 23.9 MM dry tons per year available
- In stage 2, we continued to improve our understanding of sustainable collection
  - Stage 1
  - Stage 2
    - Erosion control limits
    - Soil carbon limits



## Stage 2 findings

### Economic impacts of stover to ethanol in Iowa

- At a selling price of \$1.25 per gallon ethanol
  - 15 economically feasible plants, producing almost 1 bgy of Ethanol
  - Create 14,253 annual jobs in industrial (57%), transportation (24%) and ag sectors (19%)
  - Create \$2.4 billion in annual Total Product Output in industrial (71%), transportation (12%) and ag sectors (17%)
  - Create \$950 million annual Value Added in industrial (62%), transportation (15%) and agricultural sectors (23%)



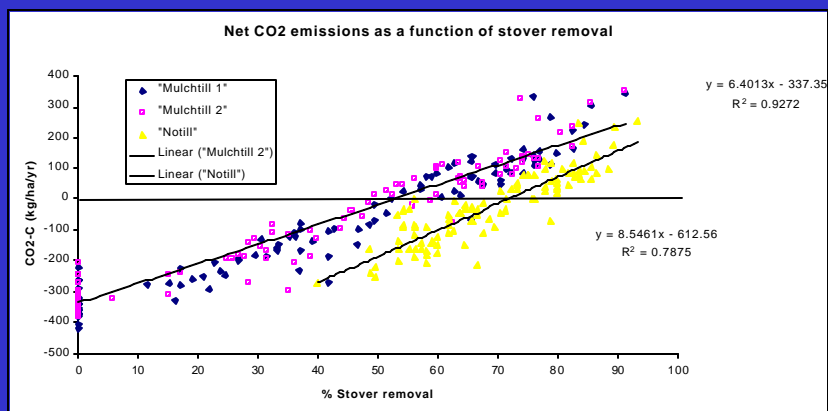
## Stage 2 findings

### Sustainability

- Four big issues have surfaced in our evaluation of corn stover as a feedstock
  - Land use
  - Soil health
  - Greenhouse gas emissions
  - Water quality
- Soil health, soil carbon levels and greenhouse gases are inextricably intertwined
- Water quality remains an unknown

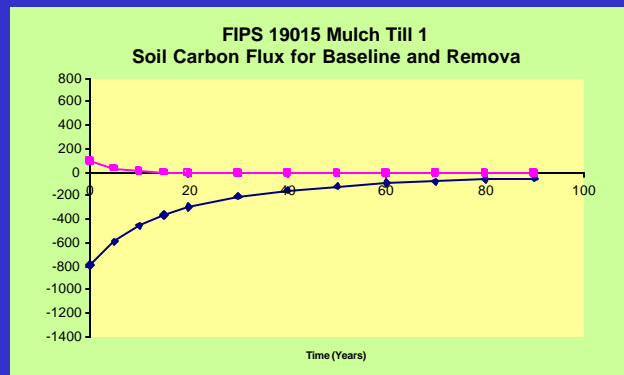
## Stage 2 findings

### Sustainability and soil carbon



## Stage 2 Findings

### Sustainability and soil carbon

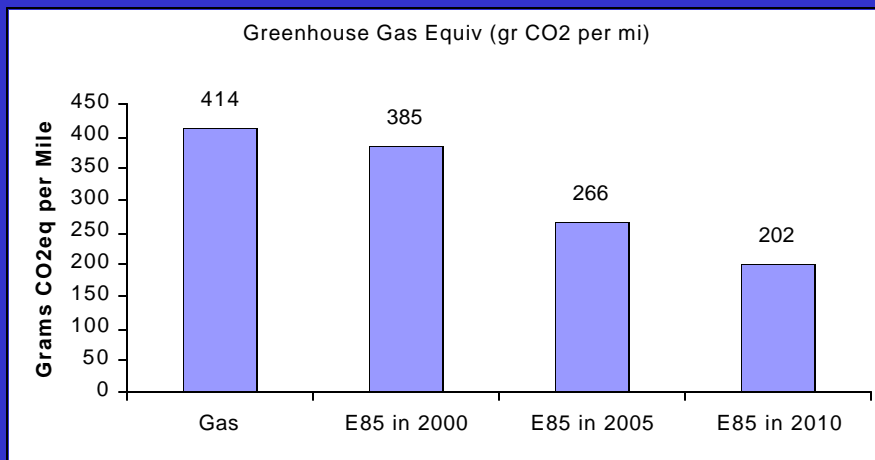


## Stage 2 Findings

### Sustainability and soil carbon

- DOE/ORNL sponsored research at USDA:
  - Lignin as a soil amendment
  - Field studies on the effects of residue removal

## Stage 2 findings Sustainability and Greenhouse Gas Effects



## Stage 2 findings Technology risks—today's approach to stover harvest

- Collect stover *after* grain harvest
- Our base case collection steps include:
  - shredding and raking in one operation
  - round baling [580 dry kg (1270 dry lb)]
  - transporting from the field to an intermediate storage facility 8 km (5 miles) away using a bale wagon pulled by a tractor
  - stacking the bales 5 high under a shed using a telescopic handler

## Stage 2 findings

### Technology risk—today's approach to stover harvest

	Low	Base	High
Yield, ton/ac	1.1	1.5	2.5
\$/ton	\$31.10	\$26.90	\$22.20
Density, lb/ft <sup>3</sup>	7	9	10
\$/ton	\$30.90	\$26.90	\$25.50
Operating hours	50%	100%	150%
\$/ton	\$29.80	\$26.90	\$25.80
Combinations*, \$/ton	\$41.00	\$26.90	\$21.00

## Stage 2—Technology Risks

### Feedstock Composition

- Corn stover is a complex material
- Economics of ethanol are highly dependent on carbohydrate and lignin content
- Rapid analysis techniques developed at NREL are leading to a more robust understanding of composition and how it will vary
- Groundbreaking analysis techniques not only help to quantify the technical issues in the development stages for a stover-based refinery, but offer vital real-time tools for use in the ethanol plant and in the field

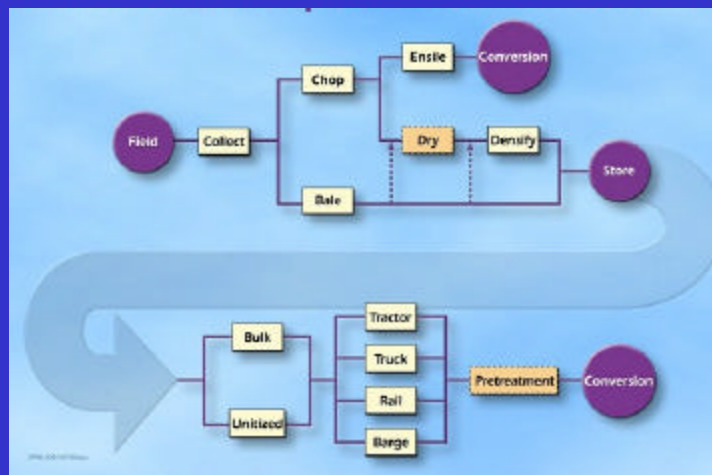
## Stage 2 findings

### Technology risks—today's approach to stover harvest

- Timing is tricky—constrained by grain harvest, stover moisture content and weather
- Inefficient
- Leads to poor quality feedstock
- In Stage 1 we had identified these problems
- In Stage 2, we focused on benchmarking the existing collection strategy and identifying improvements

## Stage 2 findings

### Improving collection technology



## Stage 2 Findings

### Improving collection technology

- International Harvester 1460 Axial Flow Combine with Row Bean Head
  - Collects the Whole Plant
  - Harvests and Separates Grain From Stover
- Stover Conveyed to Hesston Stakhand 10
  - Density of Stover Increased
  - Dirt Free Collection



## Feedstocks—what have we learned in stage 2?

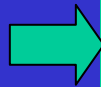
- As stage 2 comes to an end, we can conclude that:
  - Environmentally sustainable collection of stover is possible
  - There is a sufficient supply of stover to meet our projected market opportunities
  - Soil sustainability is not a showstopper
  - BUT, soil sustainability and its impact on stover cost and climate change are still poorly understood
  - There are opportunities to reduce the delivered cost of stover collection

## Feedstocks—what do we need to do in stage 3?

- Improve our understanding of soil sustainability
- Reassess the size of stover as a resource in light of improved information on soil sustainability
- Develop more efficient collection strategies that systemically reduce cost and risk
- Use life cycle analysis as a tool for dialogue about the benefits and risks of a stover-based biorefinery as a sustainable option for society

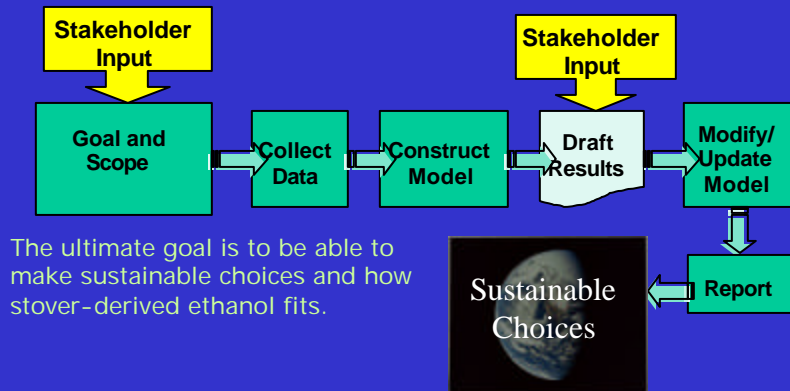
## Talking about sustainability

- “Systems oriented”
- “Expanding Resources”
- “Quality of Life”
- “Earth”
- “Ethic”



- Life Cycle
- Renewable Resources
- Economics
- Environment
- Dialogue


## Life cycle analysis—a tool for dialogue





# Competitive Technology/ Detailed Technical Assessment: **Pretreatment**

Dan Schell

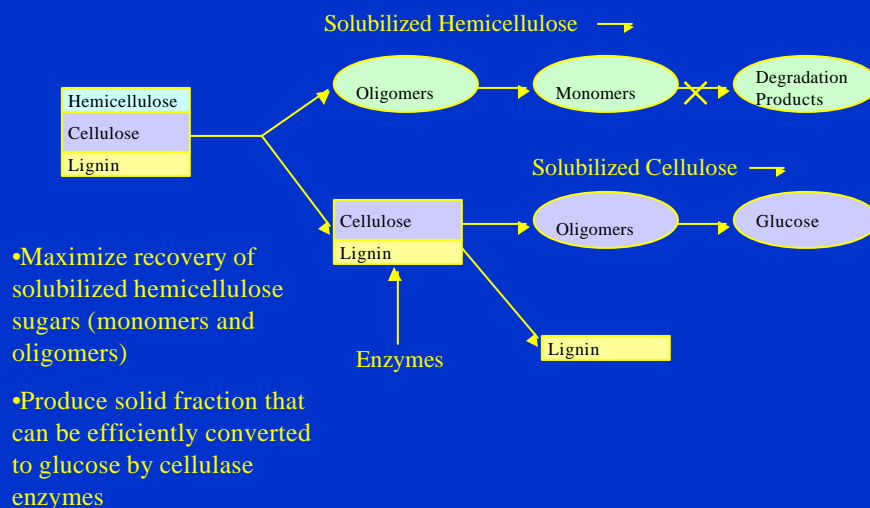
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## Presentation Outline

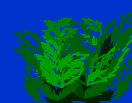
- Background
- Technology selection
  - Selection process
  - Information gathering process
  - Results
  - Recommendations
- Pretreatment technology status
  - Investigating technical feasibility
  - NREL capabilities and recent results
- Findings

# Goals of Biomass Pretreatment

## Example for an Acid-Catalyzed Process



## Pretreatment Challenges



### Challenging Reaction Chemistry and Heat and Mass Transfer Conditions

- Many options with poorly understood chemistry
- Heterogeneous solid feedstock that is difficult to chemically analyze
- Broad particulate distribution
- Achieving multiple objectives
  - Maximizing hemicellulose sugar yield
  - Maximizing cellulose conversion



## Pretreatment Challenges



### Challenging Process Conditions

- High temperature and highly corrosive environment (some options)
- High solids loading
- Understanding post-processing requirements to recover oligomeric sugars
- Understanding downstream consequences (i.e., effects on integrated performance, solid precipitates, etc.)
- Understanding environmental and safety issues

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## Pretreatment Selection

### Aggressive Project Schedule

Technology must already be undergoing performance testing/validation at the pilot scale

## Goal for Selection Effort

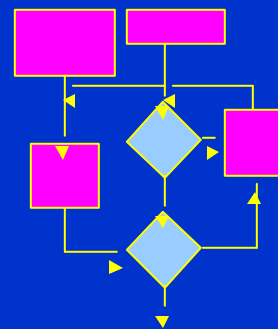
- Objective: To select from the many varieties the best technologies for process integration efforts in Stage 3.
  - Select a pretreatment now to begin stage 3 work
  - Review all technologies and make final selection by late FY02

# Variety of Biomass Pretreatments

Pretreatment Category	Pretreatment Types
Base-Catalyzed	AFEX/FIBEX
	Alkaline-Peracetic
	Alkaline-Peroxide
	Alkaline-Solvent
	Ammonia
	Lime
	Sodium Hydroxide
Non-Catalyzed	Autohydrolysis
	Comminution
	Hot Water
	Hot Water-pH Neutral
Acid-Catalyzed	Hydrochloric
	Nitric
	Peracetic
	Phosphoric
	Sulfur Dioxide
	Sulfuric
Solvent-Based	Organosolv
Chemical-Based	Solvents
	Peroxide
	Wet Oxidation
	Supercritical Carbon Dioxide
Others	Biological
	Radiation

## Selection Process

- Applied two-tiered screening process to reduce number of pretreatment options
  - First screen (technical performance)
  - Second screen: data quality and technology readiness/availability
- Biofuel Program's Advanced Pretreatment Task is tracking all pretreatments



## Criteria for First Screen

Is the pretreatment effective? Does it meet minimum performance criteria?

1. hemicellulose sugar yield (total sugars) ? 75%  
AND  
enzymatic cellulose conversion ? 80%
- OR
2. total sugar yields equivalent to 1.

## Criteria for Second Screen

Is the pretreatment sufficiently developed to consider it for Stage 3?

- Data quality criteria:
  - Performance data is required that is supported by carbon & mass balances
- Readiness criteria:
  - Pilot scale work reported and facilities available by June, 2002
  - Small amounts of pretreated material available for bench-scale testing by Dec. 2001
  - Sufficient (drum-scale) pretreated material available to meet process development needs by June 2002

# Approach to Information Gathering

- Comprehensive literature survey of pretreatment technologies
  - Applied tiered screening to identify top candidates
  - Information is available in a Microsoft Access™ database
- Obtained information from pretreatment researchers and technology developers (e.g., Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI))
  - Followed suggestions of gate 2 reviewers to engage pretreatment community
  - Presented at two CAFI meetings, sent questionnaire to the pretreatment community in September 2001, followed up with phone and emails contacts

## Questionnaire

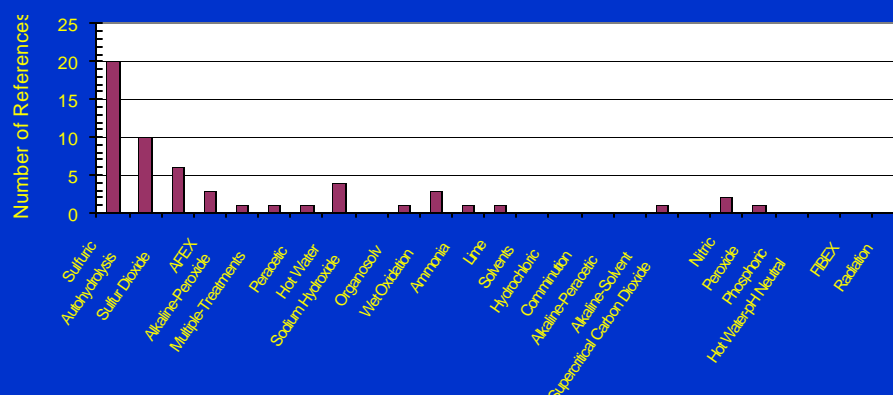
### Information Requested from Technology Developers

- Technical criteria-experimental data
  - Hemicellulose (Xylose) yield data?
  - Cellulose digestibility?
  - Ethanol yield?
  - Type of feedstock?
- Quality criteria
  - Carbon/mass balance data available?
- Readiness criteria
  - Economic analysis available?
  - Supply small quantities by Dec. 2001?
  - Supply large quantities by June 2002?
  - Where could pretreatment be performed at the pilot scale?



## Highlights from Literature Survey

- ~ 600 unique citations found from literature search and discussions with technology developers
  - New reference continue to be added to database
- 54 papers provided hemicellulosic sugar yield and cellulose conversion data



## Passing to the Second Screen

- Pretreatments from literature meeting first screen criteria
- All pretreatments being actively developed
  - Provide feedback to technology developers



## Pretreatments Undergoing Second Screen

Pretreatment Category	Pretreatments Undergoing Second Screen	Technology Developers and Providers
Base-Catalyzed	AFEX/FIBEX	Bruce Dale/Michigan State, MBI
	Ammonia*	Y.Y.Lee/Auburn
Non-Catalyzed	Lime	Mark Holtzaple/Texas A&M
	Hot Water (batch)	Charlie Wyman/Dartmouth, Mike Antal/Hawaii Natural Energy Institute
	Hot Water (percolation)	Mike Antal, Charlie Wyman
	Hot Water-pH Neutral	Michael Ladisch/Purdue
Acid-Catalyzed	Nitric Acid	Lee MacLean/HFTA
	Sulfur Dioxide	Jack Saddler/UBC, Esteban Chornet/ University of Sherbrooke
	Sulfuric Acid	BC International, Iogen, NREL, TVA, Charlie Wyman
	Sulfuric Acid (hot wash process)	NREL
	Organosolv (Clean Fractionation)	NREL
Solvent-Based		
Chemical-Based	Peroxide	
Based	Wet Oxidation	Ed Lehrburger/Pure Vision

\* Pretreatments passing first screen criteria based on literature

## Results of Selection Process

- Sulfuric acid was the only pretreatment to meet all of the first and second screen criteria
- Other pretreatments did not meet all the criteria
  - Data supported by mass/carbon balances
  - Pilot scale testing

## Recommendations

- Sulfuric acid selected for initial stage 3 process development work
- Review all technologies in late FY02, in collaboration with an industrial partner
  - Generic Aspen-based process models for acid, alkaline, and non-catalyzed processes are being developed in collaboration with technology developers
  - Technology developers to supply data

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## Pretreatment Status

### Investigating Technical Feasibility

- Assess state of dilute sulfuric acid technology
    - Exploratory pretreatment of corn stover at pilot scale
      - Supply enzyme developers with pretreated feedstock
      - Bench mark current capabilities
      - Identify showstoppers to process development
- ➡ Essential to have pretreated materials to move forward with fermentation strain screening and early integration work.

## Sunds Reactor Capabilities

Parameter	Range
Reactor Solids Concentration	19-28% (most at 20%)
Residence Times	3-12 min*
Acid Concentration	0%-5% (w/w)
Temperature	140°-195°C

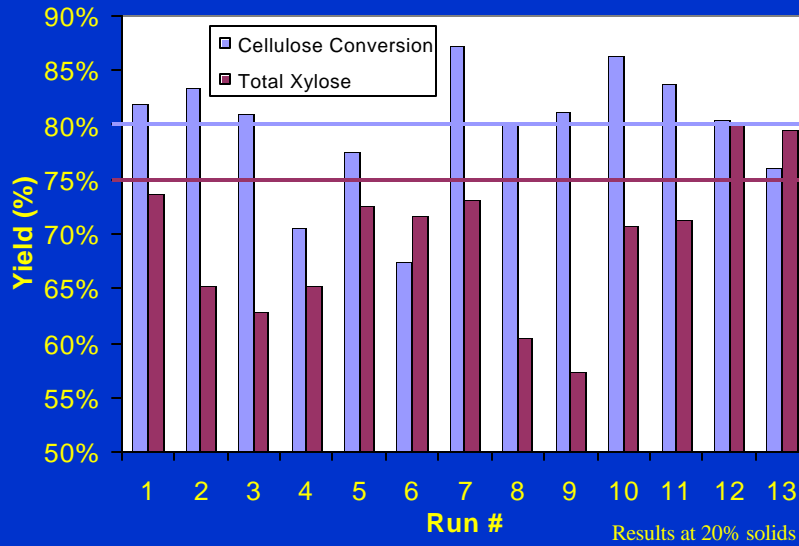
\* Flow-through operation permits a residence time of ~ 0.5-2.0 min (estimated)

- ➡ Good capability for generating pretreatment and other process residues for Biofuels Program and external client needs



## Recent Performance Results

Dilute Sulfuric Acid Pretreatment



## Improved Capability Sunds Reactor

Parameter	Can Achieve Now	With Further Optimization
Solids Concentration (%)	25-28	? 30
Total Xylose Yield (%)	80	? 85
Cellulose Conversion (%)	80	? 90

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- Background
- Technology selection
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## Findings Technology Assessment

- Dilute sulfuric acid ready for Stage 3
  - Insufficient data and/or readiness for other approaches
- Other technologies will be re-assessed if data becomes available by 8/02
  - A single pretreatment will be recommended to carry forward into FY03 work

## Findings

### Capabilities Assessment

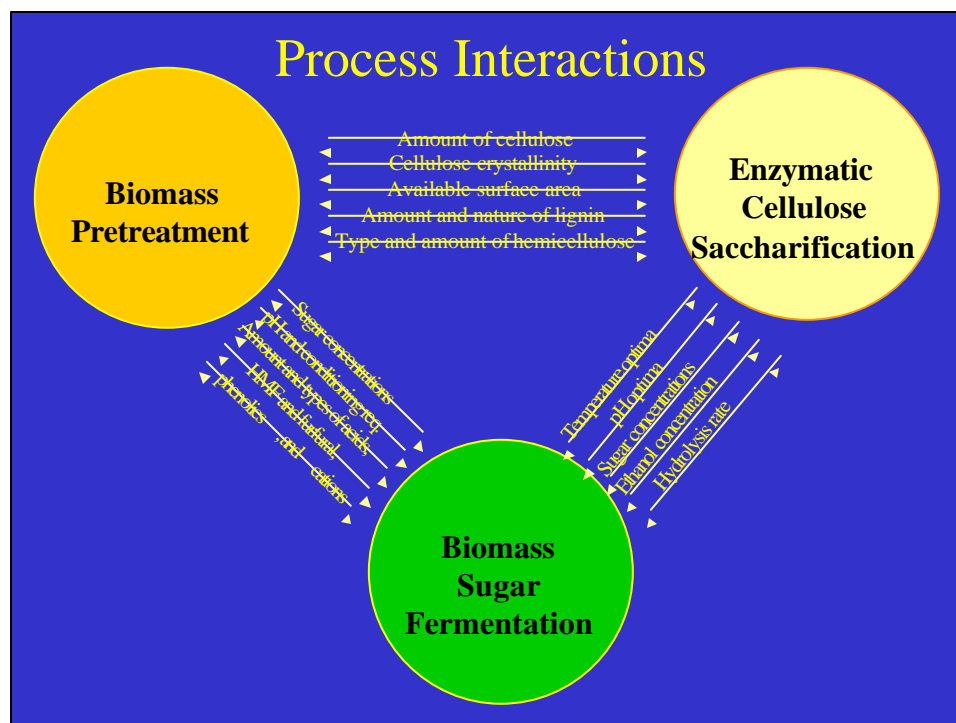
- Demonstrated 80% total xylose yield and >80% cellulose conversion at 20% solids concentration
  - Demonstrates technical feasibility
  - 28% solids concentrations has been achieved
- ? Anticipate ability to achieve critical success factors for pretreatment
- ? Stage 3 work needed to verify this

INTERMISSION  
10 minute break

# Competitive Technology/ Detailed Technical Assessment: **Enzymes**

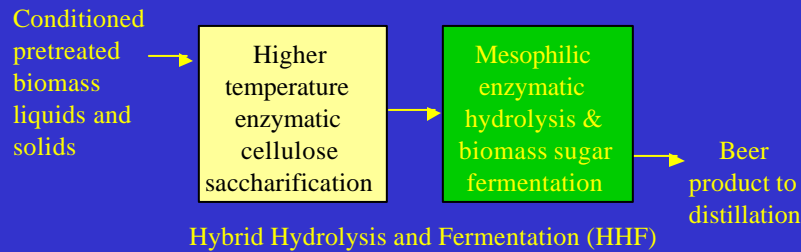
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## Outlook Favors Hybrid Configuration

- ✍ Anticipate using a hybrid hydrolysis and fermentation (HHF) process configuration that begins like SHF and ends like SSF.



- ? *Process economics will determine the most economic route. Difficult to assess before technology selection completed.*

## Enzymes — Current Status

- ✍ Next generation enzymes under development; lower cost cellulases anticipated in 2003-2004
- ✍ We will hear brief reports on the status and outlook from the enzyme developers
  - ✍ Genencor – Bill Dean and Mike Knauf
  - ✍ Novozymes – Joel Cherry



## Genencor Report

Bill Dean and Mike Knauf

## Novozymes Report

Joel Cherry

## Today

- Project Overview
- Market Assessment
- Technical and Economic Analysis
- Life Cycle Analysis
- Feedstock
- Pretreatment
- Enzyme
- ✍ **Fermentation Microorganism**
- Business plan
- High-level Stage 3 plan

# Competitive Technology/ Detailed Technical Assessment: **Fermentation Strain Selection**

Kiran Kadam

**January 30, 2002**

Operated for the U.S. Department of Energy by Midwest Research Institute • Battelle • Bechtel 

## Presentation Outline

- Background
  - Importance of effective ethanologen
  - Fermentation challenges/issues
- Strain selection
  - Methodology/Screening criteria
  - Screening results
- Recommendations for Stage 3
- Process implications
- Technical showstoppers
- Outlook for Stage 3

## Background

- Economical bioconversion of corn stover requires the following:
  - a well-pretreated substrate
  - an efficient cellulase system
  - an effective ethanologen (ethanol producing microorganism)
- Potential ethanologen
  - Important process technology component
  - Aggressive project schedule
    - Project is not developing new strains
    - Existing strains whose potential has been demonstrated

## Fermentation Challenges/Issues

- Feedstock cost: key economic factor
  - Corn stover: 37–40% six-carbon sugars, 23–25% five-carbon sugars ? need cofermenting strains
- Desired strain characteristics
  - Efficient fermentation of both C6 and C5 sugars (*cost*)
  - Hydrolyzate tolerance (*industrially relevant conditions*)
  - Nutrient requirements (*cost*)
  - Thermotolerance (*compatibility w/2<sup>nd</sup> generation thermostable enzymes*)
  - Stability under extended operation (*industrially relevant conditions*)
- Environmental permitting
  - More complicated for cofermenting recombinant strains
  - Corn-to-ethanol technology uses wild type yeasts

## Fermentation Strain Selection Objective and Approach

- Objective:  
Review available ethanologenic strains and recommend 2-4 strains for Stage 3 studies with corn stover hydrolyzate
- Approach:
  - Survey the literature on fermentative strains
  - Evaluate and compare performance based on literature data

## Survey of Reported Strains

- About 150 references scrutinized
- Culling criterion:  
Strains must have ability to ferment glucose and at least one pentose sugar
- 34 fermentative strain options identified
  - A few options based on using two microorganisms

## Fermentative Strain Options

1	<b>Bacteria</b>	20	<i>E. chrysanthemi</i> and <i>E. carotovora</i>
2	<i>E. coli</i> SL28, SL40	21	<i>E. chrysanthemi</i> B374 pZM15
3	<i>E. coli</i> KO11, SL40	22	<i>Clostridium thermosaccharolyticum</i>
4	<i>E. coli</i> FBR3 (plasmid pLO1297)		<b>Yeasts</b>
5	<i>E. coli</i> ST09, ST32	23	<i>Saccharomyces cerevisiae</i> 1400 (pLNH32)
6	<i>Klebsiella oxytoca</i> P2	24	<i>S. cerevisiae</i> 1400 (pLNH33)
7	<i>K. oxytoca</i> MSA1 (pLO1555)	25	<i>S. cerevisiae</i> 1400 424A(LNH-ST)
8	<i>K. oxytoca</i> SZ22(pCPP2006) and SZ66(pCPP2006)	26	<i>S. cerevisiae</i> YHM4 and YHM7
9	<i>K. planticola</i> ATCC 33531 pZM15	27	rDNA <i>S. cerevisiae</i>
10	<i>Zymomonas mobilis</i> 39676 (pZB206)	28	<i>S. cerevisiae</i> 424 and T1 w/ pLNH32
11	<i>Z. mobilis</i> ZM4 (pZB5)	29	<i>S. cerevisiae</i> 424A(LNH-ST)
12	<i>Z. mobilis</i> 39676 (pZB4L)	30	rDNA <i>S. cerevisiae</i>
13	<i>Z. mobilis</i> C25		<b>Fungi</b>
14	<i>Z. mobilis</i> AX101	31	<i>Fusillomyces</i> sp. NF1
15	<i>Bacillus stearothermophilus</i> pNW-PET		<b>Combinations</b>
16	<i>B. stearothermophilus</i> LLD-15, LLD-16, T13	32	<i>S. cerevisiae</i> (ATCC 600803) and <i>C. rugosa</i> V-7124
17	<i>Lactobacillus casei</i> 686 (pRSG02)	33	<i>S. mobilis</i> and <i>C. saccharolyticum</i>
18	<i>Bacteroides polypragmatus</i> Type strain GP4	34	<i>S. cerevisiae</i> and <i>C. carotivum</i>
19	<i>Erwinia chrysanthemi</i> EC 16 pLO1555		

## Methodology

- Methodology similar to that used in pretreatment selection
- Primary screen with a broad set of criteria
  - Basic efficacy screen
- Secondary screen with quantitative criteria
  - Impact on MESP
- Recommend strains for Stage 3
  - Select top ranking strains
  - Availability for licensing by a third party

## Primary Screen

- Minimum performance criteria
  - ? 80% ethanol yield on sugars
  - ? 4% (w/v) ethanol concentration
- Identify other compelling traits
  - Thermotolerance
  - Secretion of endoglucanases/hydrolytic enzymes
  - Ability to metabolize cellobiose
  - Demonstration at pilot scale

## Strains Passing Primary Screen

Strain	Ownership
<i>E. coli</i> KO11	U. of Florida/BCI
<i>E. coli</i> SL28, SL40	U. of Florida/BCI
<i>E. coli</i> FBR3 (plasmid pLO1297)	USDA/ U. of Florida/BCI
<i>K. oxytoca</i> P2	U. of Florida/BCI
<i>K. oxytoca</i> SZ2/6(pCPP2006)	U. of Florida/BCI
<i>E. chrysanthemi</i> EC 16 pLOI 555	U. of Florida/BCI
<i>Z. mobilis</i> 39676 (pZB4L)	NREL
<i>Z. mobilis</i> AX101	NREL
<i>Paecilomyces</i> sp. NF1	NREL
<i>S. cerevisiae</i> 1400 (pLNH32)	Purdue U./Iogen
<i>S. cerevisiae</i> 1400 424A(LNH-ST)	Purdue U./Iogen
<i>S. cerevisiae</i> 424A(LNH-ST)	Purdue U.
<i>S. cerevisiae</i>	U. of Stellenbosch, S. Africa
<i>S. cerevisiae</i> (ATCC 60868) and <i>P. stipitis</i> Y-7124	n/a
<i>B. stearothermophilus</i> LLD-16	Imperial College/ Agrol Technologies Ltd., UK
<i>C. thermosaccharolyticum</i>	MIT
<i>Z. mobilis</i> and <i>C. saccharolyticum</i>	n/a

## Substrate Utilization Range of Strains Passing Primary Screening

	Glucose	Xylose	Other Hexoses	Arabinose	Cellobiose
→ rDNA <i>E. coli</i>	?	?	?	?	
→ rDNA <i>K. oxytoca</i>	?	?	?	?	?
rDNA <i>E. chrysanthemi</i>	?	?	?		?
→ rDNA <i>Z. mobilis</i>	?	?		?	
→ wt <i>Paecilomyces</i> sp.	?	?	?	?	
rDNA <i>S. cerevisiae</i>	?	?	?		
wt <i>S. cerevisiae</i> and wt <i>P. stipitis</i>	?	?	?		
wt <i>B. stearo</i> thermophilus	?	?			?
wt <i>C. thermosaccharolyticum</i>	?	?			
wt <i>Z. mobilis</i> and wt <i>C. saccharolyticum</i>	?	?			

? indicates ability to ferment to ethanol; wt indicates wildtype species

## Secondary Screen

- Net change in MESP as screening criterion
- Cost impacts of key strain traits
  - Ethanol production efficiency
  - Thermotolerance
- Traits for which cost impacts not quantified:
  - Nutrient requirements
    - Estimate costs from literature but don't use results to reject strain
  - Ability to secrete endoglucanases
    - Lower enzyme loading?
  - Ability to directly metabolize cellobiose
    - Lower  $\beta$ -glucosidase requirement?



## Impacts on MESP

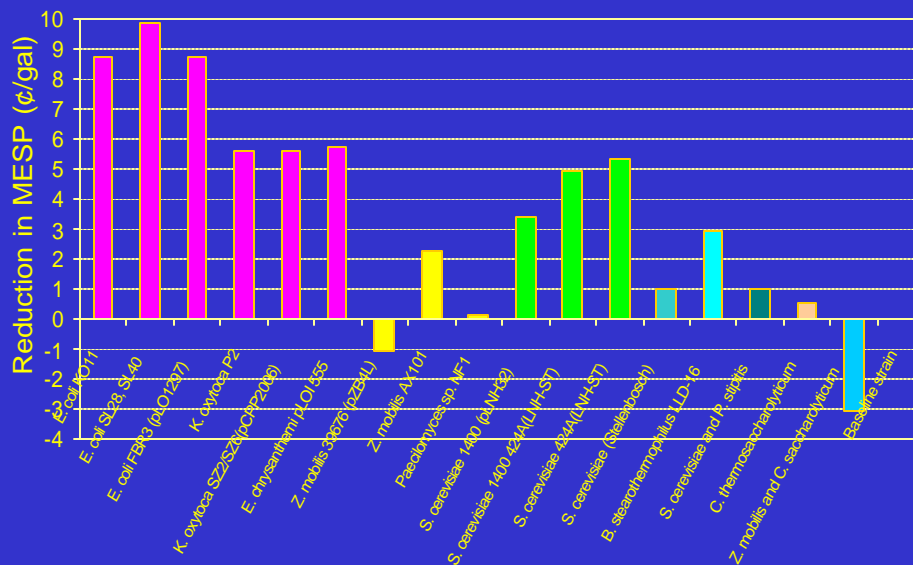
- Ethanol production efficiency

	Baseline conversion, %	¢ per additional 10% converted/unconverted to ethanol
Xylose	85	±4.0
Arabinose	85	±0.6
Galactose/mannose	0	+0.7

- Thermotolerance
  - Benefits of high-temperature SSF not quantified

Contamination loss	Cost impact, ¢/gal ethanol
5% (Baseline)	0.0
1% (Assume for thermophilic strains)	6.0

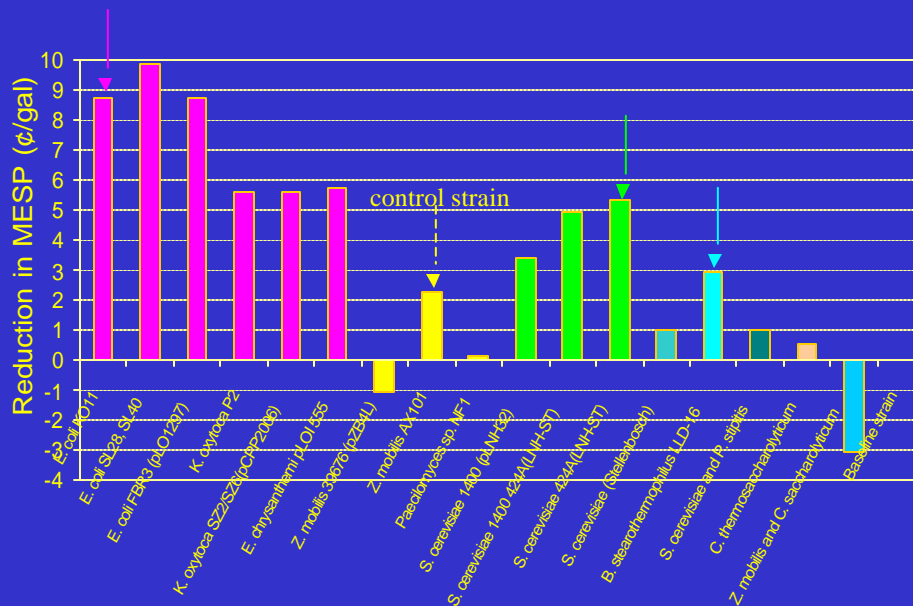
## Results of Secondary Screen



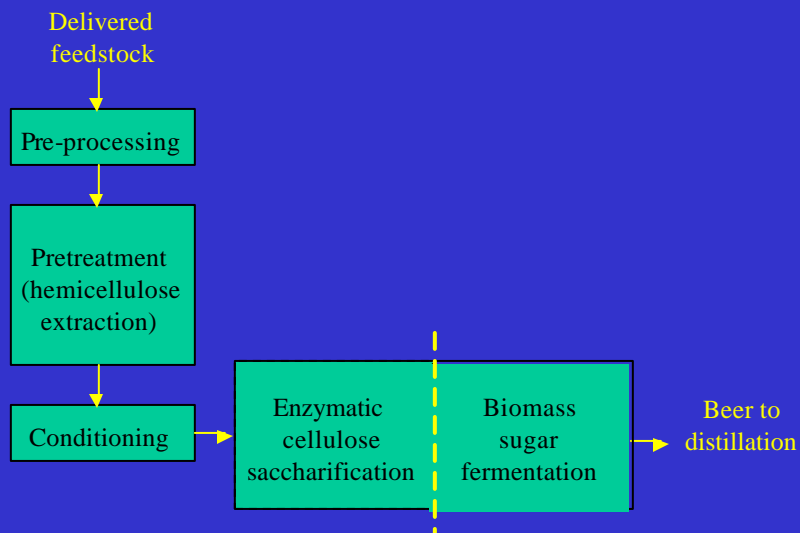
## Top Strains Passing Secondary Screen

Strain	Reduction in MESP, $\phi$ /gal ethanol	Other attributes/comments
<i>E. coli</i> KO11, SL40	9.3	Best of Ingram strains.
<i>E. coli</i> FBR3 (pLO1297)	8.8	Based on plasmid developed by U. of Florida.
<i>E. chrysanthemi</i> pLOI 555	5.7	Secretes endoglucanases and metabolizes cellobiose.
<i>K. oxytoca</i> P2	5.6	Secretes endoglucanases and metabolizes cellobiose.
<i>S. cerevisiae</i> 424A(LNH-ST)	5.4	Owned by Purdue U. Easy to license.
<i>S. cerevisiae</i> 1400 424A(LNH-ST)	4.9	Demonstrated at pilot scale. Owned by Iogen.
<i>B. stearothermophilus</i> LLD-16	2.9	Thermotolerant and metabolizes cellobiose. Nonrecombinant strain. Demonstrated at pilot scale.
<i>Z. mobilis</i> AX101	2.3	Can serve as baseline strain.

## Strains Recommended for Stage 3 Work



## Major Steps in an Enzymatic Process



## Process Implications

- pH and thermostability of cellulases being developed not yet known
- Acid or neutral cellulases best depending on strain
- Strain and enzyme characteristics affect process configuration

	pH range	Temperature range, °C	Process Implications
<i>E. coli</i> KO11	6.0–6.8	32–37	Neutral cellulases; HHF mode
<i>S. cerevisiae</i> 424A(LNH-ST)	5.2	38	Acid cellulases; HHF mode
<i>B. stearothermophilus</i> LLD-16	6.5	65–70	Neutral cellulases; HHF or SSF mode

## Technical Showstoppers

- Fermentation technology appears feasible
  - Meet aggressive conversion/rate goals set in process engineering model
    - Using realistic levels of cellulase enzyme(s)
  - “Robustness” under industrially relevant conditions
    - Demonstrate integrated process performance at a large enough scale
  - Hydrolyzate conditioning costs
    - High nutrient levels (used in reported studies) mitigate hydrolyzate toxicity
  - Nutrient costs: need to be ~3¢/gal ethanol

## Legal/Regulatory Compliance

- Patent/IP positions
  - All strain developers/owners open to third party licensing
  - Terms and agreements for such licensing need to be negotiated
- Waste streams, emissions, safety, permitting issues
  - More complicated for GMOs (genetically modified organisms)
  - But issues not insurmountable
  - Related extra costs, if any, need to be identified

## Outlook

- Early in Stage 3
  - Assess strain performance using corn stover hydrolyzate
  - Knowledge gap about sugar utilization patterns/rates re: corn stover w/minimal nutrients
  - For promising strains
    - Determine sequence of sugar consumption and rates
    - Characterize requirements for hydrolyzate conditioning
  - For the most promising strain(s)
    - Develop/demonstrate low-cost media
- Later in Stage 3
  - Optimization of HHF
  - Process integration
  - Identify terms for licensing by a third party (i.e., the technology commercializer)

Questions?

# Today

- Project Overview
- Market Assessment
- Technical and Economic Analysis
- Life Cycle Analysis
- Feedstock
- Pretreatment
- Enzyme
- Fermentation Microorganism
- ✂ **Business plan**
- High-level Stage 3 plan



## Enzyme Sugar-Ethanol Platform ***Business Plan***

James D. McMillan

Gate 3 Project Review, Golden, Colorado

January 30, 2002

Operated for the U.S. Department of Energy by Midwest Research Institute • Battelle • Bechtel



## Part III: Presentations

### ✍ Business Plan

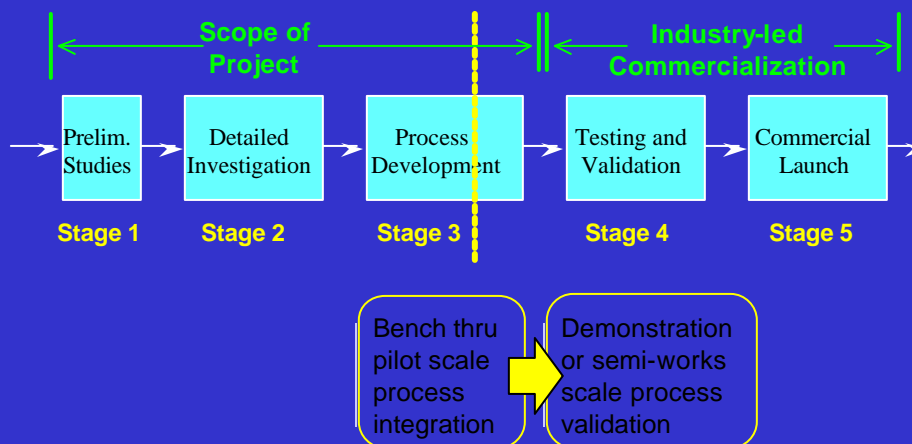
- ✍ Overall Plan – Jim McMillan
- ✍ Colloquy Results – Jim Hettenhaus (cea)
- ✍ LOI for Stage 4 Demo. Plant – John Ashworth

### ✍ Stage 3 Overview – Jim McMillan

## Overall Business Plan Outline

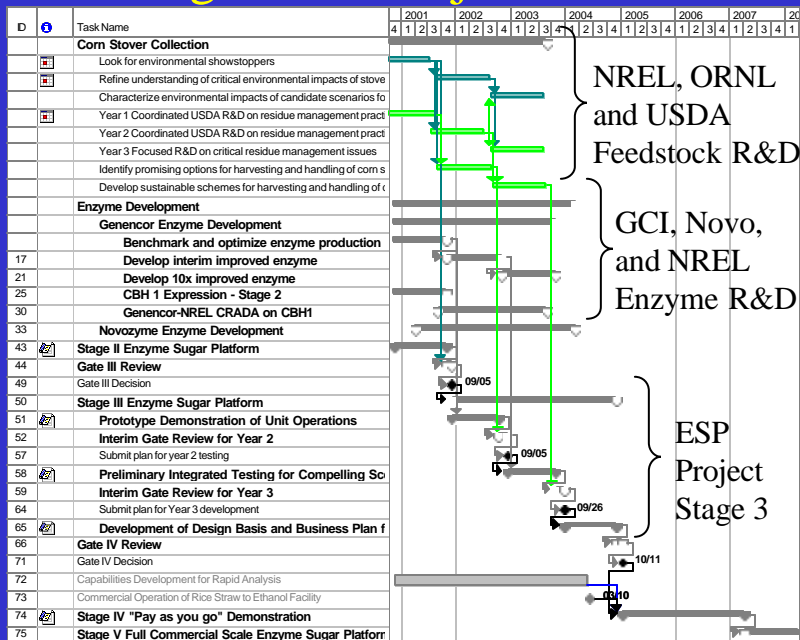
- Commercialization path
- High-level project plan
- Project coordination
- Anticipated timeline

## Commercialization Path



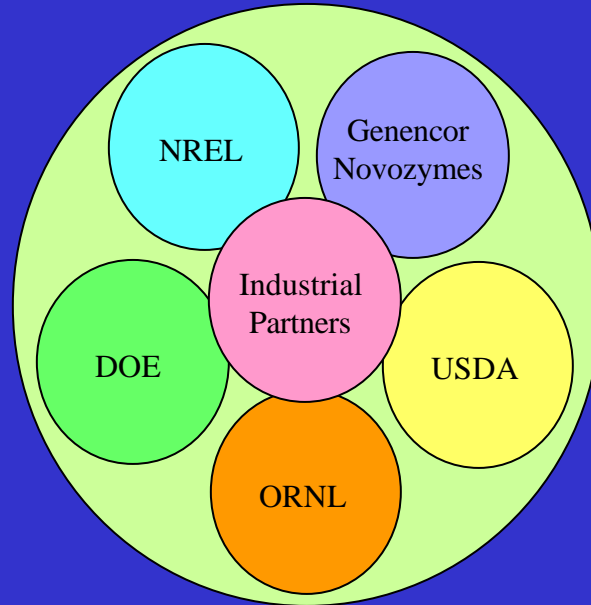
? Project success requires industry participation in Stage 3

## High-level Project Plan





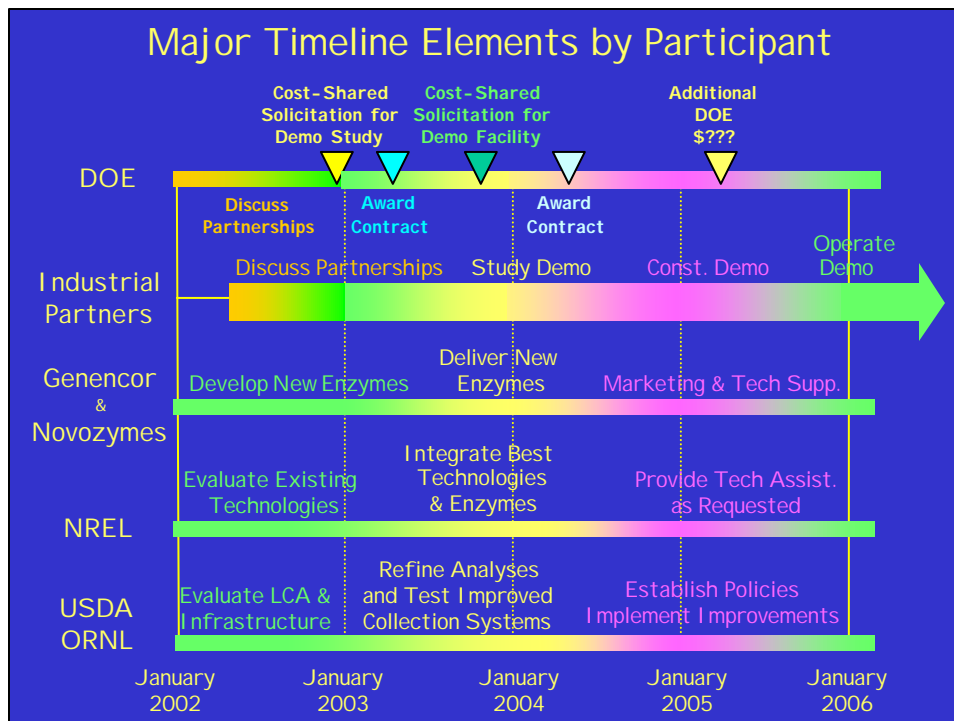
## Large Project Team



## Project Coordination

- Parallel efforts must succeed on schedule
  - Sustainability studies (LCA, etc.) must show that corn stover is a renewable and abundant feedstock
  - Collection infrastructure and policies must exist and be able to supply corn stover at low cost
  - Enzymes must be available for later Stage 3 process integration and demonstration work
- Stage 3 is a large effort
  - a variety of potential systems must be evaluated

*✍ Coordination is essential for project success!*



## Key External Tasks

- Increase rigor of sustainability analysis
  - Continue farmer and environmental community outreach
  - Create a public forum to discuss collection issues
  - Extend efforts to reduce feedstock cost
  - Begin working with USDA resource conservation districts and with non-profit groups focused on rural development
- Build support and expertise for policy development
  - Increase recognition of project's alignment with Congress's bioenergy & bioproducts R&DD goals
  - Facilitate USDA ownership of infrastructure development
  - Bring EPA into the picture to address regulatory issues
  - Demonstrate GHG reduction role for corn stover to bioenergy & bioproducts

Colloquy Results

LOI Issuance

## Today

- Project Overview
- Market Assessment
- Technical and Economic Analysis
- Life Cycle Analysis
- Feedstock
- Pretreatment
- Enzyme
- Fermentation Microorganism
- Business plan

✍ **High-level Stage 3 plan**